

Amendments to the Specification

Please amend page 6, beginning at line 25, BRIEF DESCRIPTION OF THE DRAWINGS, and ending on page 7, line 13, as follows:

BRIEF DESCRIPTION OF THE DRAWINGS

~~Figure 1 is~~ Figures 1-1A are the sequence listing for MC4R in pigs (SEQ ID NO:1). "X" represents the site of the polymorphism.

~~Figure 2 represents~~ Figures 2A-2C represent a comparison of the DNA sequence between the human (SEQ ID NO:2) and the porcine (~~SEQ ID NO:3~~ SEQ ID NO:1) MC4R gene.

~~Figure 3 represents~~ Figures 3A-3B represent a comparison of the amino acid sequence between the human (~~SEQ ID NO:4~~ SEQ ID NO:3) and the porcine (~~SEQ ID NO:5~~ SEQ ID NO:4) MC4R gene.

Figures 4a, 4b, ~~and 4c and 4d~~ are linkage reports for MC4R from CRI-MAP.

Figure 5 depicts partial nucleotide and amino acid sequences (~~SEQ ID NO:12~~ SEQ ID NOS:27-29) of the porcine MC4R gene. The amino acid translation shows an amino acid substitution at codon 298.

Figure 6 depicts multiple-alignments of the putative seventh transmembrane domain of porcine MC4R with other MCRs and GPCRs (~~SEQ ID NOS:11-26~~). The "*" represents the predicted sequence positions for porcine MC4R. The other amino acid sequences were obtained from the GenBank database (accession numbers P32245, P70596, P41983, P56451, P34974, P41968, P33033, Q01718, Q01726, Q28031, AF011466, P21554, P18089, P30680, P47211). The missense variant in porcine MC4R substituted amino acid N for D in the position marked with an arrow. The Asp (D) residue is highly conserved among MCRs, and the Asn (N) residue is well conserved in most other GPCRs.

Please amend page 8, last paragraph beginning at line 24, as follows:

Another embodiment of the invention provides a kit for assaying the presence in a MC4R gene sequence of a genetic marker. The marker being indicative of heritable traits of meat quality characteristics. The kit in a preferred embodiment also includes novel PCR primers comprising 4-30 contiguous bases on either side of the polymorphism to provide an amplification system allowing for detection of the G → A Transition polymorphism by PCR digestion of PCR products. The sequence surrounding the polymorphic site is shown in SEQ ID NO:1, Figure 1. Several primers have also been disclosed including ~~SEQ ID NOS:6 and 7, SEQ ID NOS:10 and~~

~~11- SEQ ID NOS:5 and 6, SEQ ID NOS:9 and 10 and mapping primers 8 and 9 7 and 8. The preferred primers are SEQ ID NO:10 and SEQ ID NO:11. SEQ ID NO:9 and SEQ ID NO:10.~~

Please amend page 9, second paragraph as follows:

According to the invention, in a preferred embodiment, the polymorphism in the MC4R gene identifiable by the *Taq I* restriction pattern, is disclosed. As is known in the art, restriction patterns are not exact determinants of the size of fragments and are only approximate. When the primers ~~SEQ ID NOS:6 and 7~~ SEQ ID NOS:5 and 6 are used the polymorphism is identifiable by three bands from a *Taq I* digestion of the PCR product, 466, 225, and 76 base pairs (bp) for one homozygous genotype (allele 1); two bands, 542 and 225 bp for another homozygous genotype (allele 2); and four bands for the heterozygous genotype (542, 466, 225, and 76 bp). When the preferred primers are used, ~~SEQ ID NOS:10 and 11~~ SEQ ID NOS:9 and 10 the bands upon *taq I* digestion include 156 and 70 bp for allele 1 and one 226bp fragment for allele 2. Those of skill in the art will appreciate that the design of alternate primers PCR conditions and restriction patterns for identifying the presence of allele 2 using the MC4R sequence data herein or other data for closely linked loci represent nothing more than routine optimization of parameters and are intended to be within the scope of the invention. The marker for improved meat characteristics as evidenced by all four meat quality measurements observed herein (allele 2). The allele 2 genotype was previously associated with faster growth rate. This is surprising because the current state of the art concluded that there is a negative correlation between growth rate and meat quality.

Please amend page 10, first full paragraph, as follows:

From sequence data, it was observed that in allele 2 a guanine is substituted with an adenine at position 678 of the PCR product shown in Figure 1 or at position 298 of the analogous human MC4R amino acid of the MC4R protein changing the aspartic acid codon (GAU) into an asparagine codon (AAU). The PCR test for the polymorphism used a forward primer of 5'-TGG CAA TAG CCA AGA ACA AG-3' (~~SEQ ID NO: 6~~ SEQ ID NO:5) and a reverse primer of 5'-CAG GGG ATA GCA ACA GAT GA-3' (~~SEQ ID NO: 7~~ SEQ ID NO:6). Pig specific primers used for physical mapping were a forward primer of 5'-TTA AGT GGA GGA AGA AGG-3' (~~SEQ ID NO: 8~~ SEQ ID NO:7) and a reverse primer of 5'-CAT TAT GAC AGT TAA GCG G-3' (~~SEQ ID NO:9~~ SEQ ID NO:8). The resulting amplified product of about 750 bp, when digested with *Taq I*, results in allelic fragments of 466, 225, and 76 bp (allele 1) or 542 and 225

bp (allele 2). The most preferred primers resulting in either 2 or 1 fragment after *Taq* I digestion are ~~SEQ ID NOS:10 and 11~~ SEQ ID NOS:9 and 10. Allele 1 generates fragments of 156 and 70 base pairs while allele 2 generates a single 226 bp fragment.

Please amend page 13, lines 20 and 21 as follows:

MC4R1: 5'-TGG CAA TAG CCA AGA ACA AG 3' (~~SEQ ID NO:6~~ SEQ ID NO:5)

MC4R4: 5'-CAG GGG ATA GCA ACA GAT GA 3' (~~SEQ ID NO:7~~ SEQ ID NO:6)

Please amend page 15, second full paragraph, as follows:

Figures 2 and 3 illustrate the differences between the DNA and amino acid sequences of the human and porcine MC4R gene (~~SEQ ID NOS:2-5~~ SEQ ID NOS:1-4).

Please amend page 18, second and third paragraphs, as follows:

PCR amplification of a pig MC4R gene fragment. Primers were designed from homologous regions of human and rat MC4R sequences (GenBank accession no. s77415 and u67863, respectively). The primers were: forward primer: 5'-TGG CAA TAG CCA AGA ACA AG-3' (~~SEQ ID NO:6~~ SEQ ID NO:5) and reverse primer: 5'-CAG GGG ATA GCA ACA GAT GA-3' (~~SEQ ID NO:7~~ SEQ ID NO:6). The PCR reaction was performed using 12.5 ng of porcine genomic DNA, 1x PCR buffer, 1.5 mM MgCl₂, 0.125 mM dNTPs, 0.3 mM of each primer, and 0.35 U *Taq* DNA polymerase (Promega) in a 10μL final volume. The conditions for PCR were as follows: 2 min at 94°C; 35 cycles of 30 s at 94°C, 1 min at 56°C, 1 min 30 s at 92°C, and a final 15 min extension at 72°C in a Robocycler (Stratagene, La Jolla, CA).

Sequencing and mutation detection. Sequencing of the PCR products from several individual pigs of different breeds was conducted and the sequences were compared to detect any nucleotide change. Sequencing was performed on an ABI sequencer 377 (Applied Biosystems). The porcine MC4R sequence has been submitted to GenBank, and has accession number AF087937. The sequence analysis revealed one nucleotide substitution situated within a *TaqI* restriction enzyme recognition site (Kim et al. 1999). A set of primers was then designed to generate a smaller MC4R gene fragment, which contained only one informative *TaqI* restriction site to specify the polymorphic site and to facilitate the PCR-RFLP test. These primers were:

forward 5'-TAC CCT GAC CAT CTT GAT TG-3' (~~SEQ ID NO:10~~ SEQ ID NO:9) and reverse:
5'-ATA GCA ACA GAT GAT CTC TTT G-3' (~~SEQ ID NO:11~~ SEQ ID NO:10).

Please amend page 23, line 2 as follows:

Table ~~[[1]]~~2

Least square means for different MC4R genotypic classes based on a sample of 1146 animals from six genetic lines (preferred class in bold)

Please amend page 23, the paragraph beginning at line 9, as follows:

Significant effects of marker genotype are identified for ultimate pH (pHu), color (Min) and drip loss and a desirable trend is observed for marbling. The size of the effects observed between genotypes while small are of commercial significance in terms of differences in meat quality. It can be seen from the results in Table ~~[[1]]~~2 that allele 2 is the preferred allele in this sample for all four meat quality measures. Interestingly, this is the preferred allele for growth as reported in WO 00/06777. This is a particularly important finding, as it is somewhat unexpected. In general, there is a negative correlation between growth rate and meat quality. Indeed, there is a general perception that meat quality has decreased as breeders have selected for increased growth rate.

Please amend page 24, third paragraph, as follows:

A total of 257 animals from a Pietrain-based line of pigs were slaughtered and meat quality characteristics determined at the time of slaughter and during post-slaughter handling/conditioning for meat production. MC4R genotypes were determined using methods disclosed herein. Associations between marker genotype and MQ traits were then calculated. The results are depicted in Table ~~[[2]]~~3.

Please amend page 25, line 5, as follows:

Table ~~[[2]]~~3